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(54) **METHOD OF COMBATING INFECTION**

VERFAHREN ZUR BEKÄMPFUNG VON INFEKTIONEN

METHODE PERMETTANT DE COMBATTRE UNE INFECTION

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Description

[0001] This invention relates to combating infection and in particular to the treatment of parasitic protozoal infections

[0002] The listing or discussion of a prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

[0003] The incidence of drug-resistant parasitic protozoal diseases has grown significantly in recent years resulting in an increased number of deaths in both developing countries and the Western world. Strategies being developed to address the problem include the development of new drugs, the adoption of strict treatment regimens and a comprehensive public education programme.

[0004] A possible means for delivering highly active pharmacological agents to their site of action with minimal unwanted side effects in other cells and tissues is the use of prodrugs. Prodrugs have been known for many years and are used in several medical indications. They can be defined as chemical entities which are modified by metabolic or non-metabolic systems resulting in the formation or liberation of a species with the desired pharmacological activity. In many cases, prodrug forms are used to modify drug pharmacokinetics by the alternation of a physicochemical property of the drug (such as lipophilicity). In these cases, modification of the prodrug is generally non-specific, occurring by non-enzymatic degradative processes or by the metabolic action of ubiquitous enzymes. Prodrug forms may also be used, however, to target the active pharmacological agent to specific sites only, generally by exploiting the differential distribution of enzymes capable of catalysing the prodrug modification reaction.

[0005] In the field of cancer chemotherapy activation of prodrugs by target-specific enzymes has long been a goal and many potential prodrugs have been synthesised and tested in the hope that a tumour-specific enzyme would be capable of converting them specifically into a potent anti-tumour agent.

[0006] Many researchers are still active in this area utilising prodrugs designed to be activated by a variety of enzymes including β -glucuronidase (Woessner et al (2000) Anticancer Research 20, 2289-2296), DT diaphorase (Loadman et al (2000) Biochemical Pharmacology 59, 831-837) and thymidylate synthase (Collins et al (1999) Clinical Cancer Research 5, 1976-1981).

[0007] As an alternative to this prodrug monotherapy strategy some researchers have attempted to deliver the desired enzyme to the tumour, prior to administering a prodrug. Such an approach, often termed antibody-directed enzyme prodrug therapy (ADEPT), has been disclosed in WO88/07378. In this example the enzyme is linked to a monoclonal antibody which is capable of binding to a tumour-associated antigen. In this way the enzyme is delivered to the tumour site where it can act on an appropriate prodrug. A similar strategy has been disclosed, for example, in WO 96/03151, in which the gene encoding an enzyme is delivered to the tumour and, once present, expresses the desired enzyme. This strategy is often termed gene-directed enzyme prodrug therapy (GDEPT).

[0008] It is an important tenet of many of these targeting strategies that the enzyme delivered to the tumour site should not be endogenous to the host (Aghi et al (2000) J Gene Med 2, 148-164). The presence of endogenous enzymes would lead to non-specific activation of the prodrugs and toxicity to normal tissues. Accordingly, the majority of these enzyme-prodrug therapy studies have been carried out using bacterial enzymes such as carboxypeptidase G2, β -lactamase and nitroreductase.

[0009] Whilst much of the work to date in the field of site-specific prodrug activation has concentrated on anticancer therapy, there have been recent efforts to adapt this technology for use in antibacterial applications. For example, WO 99/32113 describes the use of prodrugs consisting of a cytotoxic moiety and a β -lactam moiety. In Gram-negative bacteria containing β -lactamase enzymes within the periplasmic space, the prodrug is cleaved to release the cytotoxic moiety which then goes on to disrupt vital cell functions. These prodrugs are limited in their breadth of applicability, however, in that not all bacteria express β -lactamases and in that Gram-positive bacteria tend to excrete β -lactamases such that much of the beneficial toxin-locating effect of the prodrug will be lost.

[0010] In Smyth et al ((1998) J. Org Chem. 63, 7600-7618), the S-aminosulfenimino-penicillins are introduced. These compounds are both β -lactamase inhibitors and potential prodrug templates for the delivery of a variety of agents into β -lactamase-positive bacteria. As with the β -lactam-derivatives discussed above, however, these compounds are likely to be of limited utility in Gram positive bacteria and, due to their slow permeation through outer membrane porin structures, may need to be present at undesirably high extracellular concentrations in order to achieve significant effects in Gram-negative bacteria.

[0011] A departure from the potential shortcomings of β -lactamase-dependent prodrugs has been presented by Wei and Pei ((2000) Bioorg. Med. Chem. Lett. 10, 1073-1076). These workers conceptually demonstrated the use of 5'-dipeptidyl derivatives of cytotoxic antibacterial agents as prodrugs activatable by bacterial peptide deformylase. Preliminary results using these prodrugs suggested rather weak antibacterial activity, however, and it was hypothesised that this may have been due to poor uptake of the compounds into cells.

[0012] Human parasitic diseases are endemic in many parts of the world. For example, leishmaniasis (a parasitic disease caused by an obligate intracellular protozoan transmitted by the bite of some species of sand flies) is found in approximately 90 tropical and subtropical countries around the world and in southern Europe. More than 90% of the

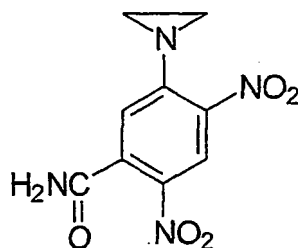
world's cases of cutaneous leishmaniasis are in Afghanistan, Algeria, Brazil, Iran, Iraq, Peru, Saudi Arabia, and Syria. However, approximately 75% of the cases that are evaluated in the United States were acquired in Latin America, where leishmaniasis occurs from northern Mexico (occasionally in rural southern Texas) to northern Argentina. More than 90% of the world's cases of visceral leishmaniasis occur in Bangladesh, Brazil, India, Nepal, and Sudan. Similarly, the geographical distribution of Chagas' Disease (caused by a flagellate protozoan parasite, *Tiypanosoma cruzi*, transmitted to humans by triatomine insects) extends from Mexico to the south of Argentina. The disease affects 16-18 million people and some 100 million, i.e. about 25% of the population of Latin America, is at risk of acquiring Chagas' disease. Even people staying for a short time in parasite-endemic areas can become infected and parasitic diseases are becoming a problem in the developed world as a result from the increase in global travel.

[0013] Resistance to the presently used drugs has been reported. Problems of resistance require the use of more toxic drugs and as the drugs are becoming less and less effective new drug discovery is needed.

[0014] The inventors have now found that, surprisingly, nitroreductase enzymes also appear to be expressed in certain parasitic protozoal organisms and the inventors suggest that tretazicar may be highly effective against parasitic infestation in animals (such as humans) because the animal (eg human) host is insensitive to this agent while the parasite will be toxically affected. The inventors have now shown that tretazicar is extremely effective against certain protozoal parasites and so it is an object of the invention to provide tretazicar as a highly effective anti-parasitic agent.

[0015] An aspect of the invention provides a use of tretazicar in the manufacture of a medicament for combating a parasitic protozoal infection of an animal.

[0016] The compound tretazicar is (5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB1954)), whose structure is shown below. Tretazicar has been used previously as an anticancer agent.



[0017] Tretazicar has the registry number C.A.S. No 21919-05-1 and its synthesis has been described in Khan & Ross (1969/70) "Tumour growth inhibitory nitrophenylaziridines and related compounds: structure-activity relationships" Chem-Biol Interactions 1, 27-47 and in Cobb et al (1969) Biochem. Pharmacol. 18, 1519-1527.

[0018] This compound is capable of eradicating a specific rat tumour ('Walker tumour'), though has little or no effect upon a variety of other tumours (Cobb et al (1969) Biochem. Pharmacol. 18, 159-1527) and shows no therapeutic benefit in clinical oncology studies (Knox et al (1993) Cancer and Metastasis Rev. 12, 195-212). It has been shown that a nitroreductase enzyme present in the Walker tumour is capable of activating CB1954 by reducing its 4-nitro group to form the compound 5-aziridino-4-hydroxylamino-2-nitrobenzamine, a potent electrophilic DNA cross-linking agent in the presence of intracellular thioesters (Knox et al (1988) Biochem. Pharmacol. 37, 4661-4669; Knox et al (1991) Biochem. Pharmacol. 42, 1691-1697). The corresponding enzyme in human cells has relatively slow kinetics of reduction, thus rendering human cells insensitive to the effects of CB1954 (Boland et al (1991) Biochem. Pharmacol. 41, 867-875).

[0019] In mechanistic studies in bacteria, it has been shown that CB 1954 has attenuated toxicity in nitroreductase-negative strains (Venitt and Crofton-Sleigh (1987) Mutagenesis 2, 375-381).

[0020] Tretazicar is activated to form an antiparasitic agent by protozoal parasite-associated enzymes. The term "protozoal parasite-associated enzyme" means an enzyme or isoform thereof which is either specific to the protozoal parasite constituting the infection, or is expressed in a functional form to such a low extent by the host organism as to render any activation of tretazicar by the latter insufficient to cause unacceptable host toxicity, but which enzyme is expressed by the protozoal parasite.

[0021] Typically, the parasite-associated enzyme is at least 10-fold or 20-fold or 50-fold or 100-fold or 500-fold or 1000-fold more active at activating the compound than an enzyme present in the host organism.

[0022] It will be appreciated that tretazicar is substantially unchanged by enzymes endogenous to the host organism and is activated substantially by one or more enzymes in the protozoal parasite.

[0023] By "activated to form an antiparasitic agent" we include the meaning that tretazicar is converted to a form which is cytotoxic, particularly to the protozoal parasite.

[0024] Similarly, it will be appreciated that the methods and medicaments of the invention are particularly suited to combat protozoal parasite infection wherein the protozoal parasite is one which contains an enzyme system which is

able to activate tretazicar into a substantially cytotoxic form. A method for determining whether a protozoal parasite is responsive to tretazicar is described in the Example. Such protozoal parasites may be killed by tretazicar. Suitably, protozoal parasitic infections which may be treated with tretazicar are ones for which tretazicar has an IC₅₀ of less than 10 micromolar preferably less than 5 micromolar and more preferably less than 1 micromolar.

[0025] Other methods of determining if a protozoal parasite contains an enzyme capable of activating tretazicar to a substantially cytotoxic form will be known to those skilled in the art. These include, but are not limited to, the use of suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group, to compare the gene sequence of the protozoa with that of known nitroreductase-containing species or the use of classical techniques whereby the relevant enzyme is detected, isolated and purified prior to testing with tretazicar,

[0026] Tretazicar is believed to be capable of covalently cross-linking protozoal parasite nucleic acid once it has been activated by an enzyme system present in the parasite to the cytotoxic form. Since tretazicar only becomes so capable on activation by a parasite-associated enzyme, the presence of tretazicar in host cells is believed not to pose a danger since they do not possess compatible enzyme activity. Binding of tretazicar to host cell components via the aziridine (or mustard) group, will present only a minor risk of cell disruption since any mono-functionally bound compound is believed to be excisable by host repair enzymatic processes (Knox *et al* (2003) *Current Pharmaceutical Design* 9, 2091-2104).

[0027] The parasite-associated enzyme responsible for activating the compound has, we believe, nitroreductase activity, for example under both oxic and hypoxic conditions.

[0028] In one embodiment, tretazicar may be used to selectively inhibit those parasites which have developed resistance to one or more currently used antibiotics.

[0029] In one embodiment of the invention, the animal is also administered one or more other compounds known to be of use in combating a parasitic infection.

[0030] Tretazicar and one or more other compounds as stated may be administered together or sequentially.

[0031] Compounds known to be of use in combating a parasitic infection include Misonidazole, nitroheterocyclics such as Nifurtimox and RSU 1069, Benznidazole antimonials such as stibogluconate, and acetylcholine derivatives such as Miltefosine.

[0032] Thus, further aspects of the invention provide use of a combination of tretazicar and one or more other compounds known to be of use in combating a parasitic infection of an animal in the manufacture of a medicament for combating a parasitic infection of an animal; and use of tretazicar in the manufacture of a medicament for combating a parasitic infection of an animal, wherein the animal is administered one or more other compounds known to be of use in combating a parasitic infection of an animal; and use of one or more other compounds known to be of use in combating a parasitic infection of an animal in the manufacture of a medicament for combating a parasitic infection of an animal, wherein the animal is administered tretazicar.

[0033] The methods and medicaments of the invention find particular utility in combating infections with one or more of *Trypanosoma cruzi*, *T. brucei*, *Leishmania* spp. particularly *L. infarutum*, *Cryptosporidium* spp. and *Giardia* spp..

[0034] A further aspect of the invention provides the combination of tretazicar with one or more other compounds known to be of use in combating a protozoal parasitic infection of a host organism.

[0035] The combination may be packaged and presented for use in medicine.

[0036] In a further embodiment, the combination may be further combined with a pharmaceutically acceptable carrier in order to form a pharmaceutical composition. A pharmaceutical composition may include, for example, tretazicar and sterile, pyrogen-free water. Typically, the pharmaceutical composition is in a liquid form in polyethyleneglycol/N methyl pyrrolidone (PEG/NMP) diluted with saline (Chung-Faye *et al* (2001) *Clinical Cancer Research* 7, 2662-2668).

[0037] A preferred embodiment is a pharmaceutical composition for oral administration. Conveniently, the pharmaceutical composition is a gelatine capsule containing the tretazicar. Typically, the pharmaceutical composition is a capsule or tablet which allows enteric release, for example by virtue of a coating which dissolves in the intestine. Methods of making such capsules and tablets are well known in the art.

[0038] The one or more compounds as defined may be administered to the host organism in any suitable form and in any amount effective to combat the infection. Suitably, the veterinary (in the case of non-human animals) or medical (in the case of humans) practitioner can select the appropriate route of administration and the appropriate dose or dosing regime. An amount of tretazicar is administered, either as single or multiple doses, in an amount effective to combat the parasitic infection.

[0039] For administration to an animal (including human), appropriate routes of administration include but are not limited to intravenous, transdermal and by inhalation. Typically, for administration to a human by infusion, the tretazicar would be administered at a dose of up to 30 mg/m². Oral administration is also suitable, such as using a gelatine capsule as discussed above.

[0040] The tretazicar may be given by a variety of routes depending on the nature and location of the infective agent. Accordingly, a variety of compositions of different pharmaceutical form are provided, as would be clear to one skilled in the art.

[0041] The invention will now be described in more detail by reference to the following Figures and non-limiting example.

[0042] Figure 1 shows the activity of tretazicar against *L. donovani* HU3 in BALB/c mice (daily IP administration).

[0043] Figure 2 shows the activity of tretazicar against *L. donovani* HU3 in BALB/c mice (daily IV administration).

[0044] Figure 3 shows the activity of tretazicar against *L. donovani* HU3 in SCID mice (daily IV administration).

Example 1: Antiparasitic activity of tretazicar

In vitro activity

[0045] Tretazicar was screened against a number of parasitic organisms using an integrated *in vitro* screening system and compared against the treatment of choice against that organism (Table 1).

[0046] As shown in Table 1, tretazicar is extremely active against *Leishmania infantum* and *Trypanosoma cruzi* and is much more active than the established agents. Cytotoxicity assays against a range of human cell lines always give an IC₅₀ value >50 μM and the therapeutic ratios against these two organisms is thus dramatic (>16,000 for *T. cruzi* and >625 for *L. infantum*). Although not as potent as suramin, tretazicar was active against *T. brucei* with a therapeutic ratio >10. No activity was seen against the tested strains of *T. colubriformis* or *Plasmodium falciparum* presumably because they do not express an enzyme system which is able to activate tretazicar into a cytotoxic form.

[0047] Given the proven clinical acceptability of tretazicar, this agent represents a novel prodrug approach for the treatment of parasitic infestation, in particular leishmaniasis and Chagas' disease.

Table 1. *In vitro* data for tretazicar against certain parasites

	<i>T. cruzi</i> (tulahuen CL2), IC ₅₀ μM
Nifurtimox	0.45
Tretazicar	0.003
	<i>L. infantum</i> , IC ₅₀ μM
Stibogluconate	6.0
Tretazicar	0.08
	<i>T. b. brucei</i> , IC ₅₀ μM
Suramin	0.045
Tretazicar	5.0
	<i>T. colubriformis</i> , IC ₅₀ μM
Albendazole	0.002
Tretazicar	>32
	<i>P. falciparum</i> (Ghana), IC ₅₀ μM
Chloroquine	0.018
Tretazicar	>32

In vivo activity

[0048] *In vivo* activity of tretazicar against *L. donovani* was tested in both BALB/c and SCID mice. The infection and treatment of the animals was performed as described by Croft and Yardley and references there in (Croft & Yardley (1999) Animal models of visceral leishmaniasis. In Handbook of Animal Models of Infection, Zak, O. (ed) pp 783-787, Academic Press, London). At the end of treatment the mice were weighted to give an estimation of drug toxicity. The liver was removed from freshly sacrificed animals and weighed. Smears were then prepared from the livers on microscope slides and fixed with methanol and stained with Giemsa stain. The number of parasites per 500 liver cells was determined microscopically for each experimental animal. This figure is multiplied by total liver weight (mg) and this figure (the

Leishman-Donovan unit (LDU)) is used as the basis for calculating the difference in parasite load between treated and untreated animals (Croft & Yardley, 1999 *supra*).

[0049] As shown in Figures 1 to 3, there is a dramatic log/linear dose response of inhibition of parasite numbers with tretazicar concentration. No drug related toxicity, as measured by body weight loss, was observed in these experiments and significant toxicity was not observed until a dose of 10 mg/kg x 5 was employed. ED₅₀ values obtained are shown in Table 2. The equivalent activity of tretazicar in both BALB/c and immunodeficient SCID mice shows that the therapy is not immune dependent. This would be predicted from the proposed mechanism of action. Stibogluconate at its MTD (15 mg/kg x 5 SC) was used as the control compound for these experiments. This dose only achieved a 52±15.2% inhibition in parasite counts in BALB/c mice and little effect in the SCID mouse model. The *in vivo* efficacy of stibogluconate is known to be T cell dependent (Murray et al (1993) Antimicrob. Agents Chemother. 37, 1504-1505). The activity of tretazicar is notably higher than standard anti-leishmanial drugs in mouse models (compare data in Table 1 with that in Croft & Yardley, 1999 *supra*).

[0050] Tretazicar is also effective against *T. cruzi in vivo*. As shown in Table 3, infected but untreated BALB/c mice only survived for an average of 13.8 days. At a dose of 0.3 mg/kg (IP x 5, daily) all the mice survived for the 50 days before the experiment was terminated. However at this dose parasites could still be detected in the blood at 13 days. A dose of 3.0 mg/kg (IP x 5, daily) cleared all the parasites from the blood. A dose of 45 mg/kg (p.o. x 5 daily) of benznidazole was required to produce an equivalent effect.

Table 2. Activity of Tretazicar against *L. donovani* HU3

Mouse Strain	Treatment	ED ₅₀ mg/Kg	ED ₉₀ mg/Kg
BALB/c	IP x 5 Daily	0.23	1.41
BALB/c	IV x 3 Daily	0.19	1.15
SCID	IV x 5 Daily	0.18	0.35

Table 3. Activity of Tretazicar against *T. cruzi*.

				Days post infection & data																	
Group #	compound ID	dosing regimen	Mice #	6	7	8	9	10	11	12	13	14	15	16	25	51					
				14/03	15/03	16/03	17/03	18/03	19/03	20/03	21/03	22/03	23/03	24/03	03/04	27/04					
				# of trypanfield, X = day of death																	
1	untreated control		1	0/20							1/10			X							
			2	0/20							35/10		X								
			3	3/20							X										
			4	2/20							X										
			0	1/20						X											
2	benznidazole	45mg/kg p.o. x5	1	0/20							0/10										
			2	3/20							0/10										
			3	3/20							0/10										
			4	4/20							0/10										
			0	1/20							0/10										
10	Vehicle alone	i.p. x5	1	1/20							X										
			2	0/20					X												
			3	2/20						X											
			4	2/20					X												
			0	0/20						2/5		X									
11	Tretazicar	0.3mg/kg i.p. x5	1	2/20							16/5				X						
			2	0/20							2/5										
			3	0/20							2/5										
			4	0/20							7/5										
			0	0/20							8/5										
12	Tretazicar	1mg/kg i.p. x5	1	0/20							0/10										
			2	0/20							0/10										
			3	0/20							3/10										
			4	1/20							X										
			0	1/20						X											
13	Tretazicar	3mg/kg i.p. x5	1	5/20							0/10										
			2	12/20							0/10										
			3	4/20							0/10										
			4	1/20							0/10										
			0	1/20							0/10										
14	Tretazicar	10mg/kg i.p. x5	1	0/20							0/10										
			2	1/20							0/10										
			3	0/20							0/10										
			4	0/20							0/10										
			0	2/20							0/10										

[0051] Given the proven clinical acceptability of tretazicar, this agent represents a novel prodrug approach for the treatment of parasitic infestation, in particular leishmaniasis and Chagas' disease.

In vitro screening methods

Chagas' disease: *in vitro* screening model *T. cruzi* (MHOM/CL/00/Tulahuen); *T. cruzi* (MHOM/BR/00/Y)

Parasite and cell cultures

[0052] The *Trypanosoma cruzi* (MHOM/CL/00/Tulahuen) transfected with β -galactosidase (Lac Z) gene, was used (Buckner *et al* (1996) *Antimicrobial Agents and Chemotherapy* 40(11), 2592-2597. The strain was maintained on an L-6 (rat skeletal myoblast cell line obtained from European Collection of Animal Cell Cultures (ECACC, Salisbury, UK)) cell-layer in RPMI 1640 w/o phenol red medium supplemented with 10% heat inactivated fetal calf serum. All cultures and assays were conducted at 37°C under an atmosphere of 5% CO₂ in air.

Drug sensitivity assays

[0053] Stock tretazicar solutions were prepared in 100% DMSO (dimethylsulfoxide) at 20 mg/ml. The stocks were kept at room temperature in the dark prior to use. For the assays, the compound was further diluted to the appropriate concentration using complete medium.

[0054] Assays were performed in sterile 96-well microtiter plates, each well containing 100 μ l medium with 2x10³ L-6 cells. After 24 hours 50 μ l of a trypanosome suspension containing 5 x 10³ trypomastigote bloodstream forms from

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culture was added to the wells. 48 hours later the medium was removed from the wells and replaced by 100 µl fresh medium with or without a serial drug dilution. After 72 hours of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterility, and to determine the minimum inhibitory concentration (MIC): this is the lowest drug concentration at which no trypanosomes with normal morphology as compared to the control wells can be seen. Nifurtimox was used as the reference drug.

[0055] The substrate CPRG/ Nonidet (50 µl) was added to all wells. A colour reaction became visible within 2-6 hours and was read photometrically at 540nm. The results, expressed as % reduction in parasite burdens compared to control wells, were transferred into a graphic programme (EXCEL), sigmoidal inhibition curves determined and IC₅₀ values calculated.

Primary screen

[0056] The compounds were tested in triplicate at 4 concentrations (30 - 10 - 3 - 1 µg/ml). Nifurtimox was included as the reference drug.

[0057] The compound is classified as inactive when the IC₅₀ is higher than 15 µg/ml. When the IC₅₀ lies between 15 and 5 µg/ml, the compound is regarded as being moderately active. When the IC₅₀ is lower than 5 µg/ml, the compound is classified as highly active and is further evaluated in a secondary screening.

Secondary screen

[0058] The same protocol was used and the IC₅₀s determined using an extended dose range adjusted as appropriate.

Leishmaniasis: *in vitro* screening

Parasite and cell cultures

[0059] One strain of *Leishmania* spp. (*Leishmania donovani* MHOM/ET/67/L82, also known as LV9,HU3) was used. The strain is maintained in the Syrian Hamster (*Mesocricetus auratus*). Amastigotes were collected from the spleen of an infected hamster and spleen parasite burden was assessed using the Stauber technique.

[0060] Primary peritoneal mouse (CD1) macrophages were collected 1 or 2 days after a macrophage production stimulation with an i.p. injection of 2ml 2% soluble starch. All cultures and assays were conducted at 37°C under an atmosphere of 5% CO₂.

Drug sensitivity assays

[0061] 20 mg/ml tretazicar stock solution was prepared in 100% DMSO and was kept at room temperature in the dark. The stock was pre-diluted to 60 µg/ml in RPMI 1640 +10% heat inactivated fetal calf serum. Assays were performed in sterile 16-well tissue culture slides, each well containing 50 µl of the compound dilutions together with 100 µl of macrophage/parasite inoculum (4x10⁵ macrophages/ml and 4x10⁶ parasites/ml). The inoculum was prepared in RPMI-1640 medium, supplemented with 10% heat inactivated fetal calf serum. Parasite growth was compared to control wells (100% parasite growth). After 5 days of incubation, parasite growth was microscopically assessed after staining the cells with a 10% Giemsa solution. The level of infection/well was evaluated by counting the number of infected macrophages per 100 macrophages. The results expressed as % reduction in parasite burden compared to control wells, were transferred into a graphic programme (EXCEL), sigmoidal inhibitions curves determined and IC₅₀ values calculated.

Primary screen

[0062] The compounds were tested in quadruplicate at 4 concentrations (30 - 10 - 3 - 1 µg/ml). Pentostam®(sodium stibogluconate) was included as the reference drug.

[0063] The compound is classified as inactive when the IC₅₀ is higher than 15 µg/ml. When the IC₅₀ lies between 15 and 5 µg/ml, the compound is regarded as being moderately active. When the IC₅₀ is lower than 5 µg/ml, the compound is classified as highly active and is further evaluated in a secondary screening.

Secondary screen

[0064] The same protocol was used and the IC₅₀ determined using an extended dose range adjusted as appropriate. Pentostam® was included as the reference drug.

Claims

1. Tretazicar for use in combating a parasitic protozoal infection of an animal.
- 5 2. Use of tretazicar in the manufacture of a medicament for combating a parasitic protozoal infection of an animal.
3. Use according to Claim 2, or tretazicar for use according to Claim 1, wherein the animal is a mammal.
- 10 4. Use or tretazicar for use according to Claim 3, wherein the mammal is a human.
5. Use or tretazicar for use according to any one of the preceding claims wherein the parasite causing the infection is associated with an enzyme system capable of activating tretazicar into a cytotoxic form.
- 15 6. Use or tretazicar for use according to Claim 5, wherein the enzyme is a nitroreductase.
7. Use or tretazicar for use according to any one of the preceding claims, wherein the animal is one which does not contain endogenously an enzyme system which activates tretazicar to such an extent as to cause unacceptable toxicity to the animal.
- 20 8. Use or tretazicar for use according to any one of the preceding claims wherein the parasite causing the infection is antibiotic resistant.
9. A use according to Claim 2 or tretazicar for use according to Claim 1, wherein the parasitic infection of the animal is caused by any one or more of the following parasites: *Trypanosoma cruzi*, *T. brucei*, *Leishmania spp.* particularly *L. infantum*, *Cryptosporidium* and *Giardia spp.*
- 25 10. Tretazicar for use according to Claim 1 wherein the animal is also administered one or more other compounds known to be of use in combating a parasitic infection.
- 30 11. Use of a combination of tretazicar and one or more other compounds known to be of use in combating a protozoal parasitic infection of an animal in the manufacture of a medicament for combating a parasitic infection of an animal.
12. Use of tretazicar in the manufacture of a medicament for combating a protozoal parasitic infection of an animal, wherein the animal is administered one or more other compounds known to be of use in combating a parasitic infection of an animal.
- 35 13. Use of one or more other compounds known to be of use in combating a protozoal parasitic infection of an animal in the manufacture of a medicament for combating a parasitic infection of an animal, wherein the animal is administered tretazicar.
- 40 14. A combination of tretazicar and one or more other compounds known to be of use in combating a protozoal parasitic infection of a host organism.
15. A combination according to Claim 14 wherein the host organism is an animal.
- 45 16. A combination according to Claim 15 for use in medicine.
17. A pharmaceutical composition comprising the combination as defined in Claim 15 and a pharmaceutically acceptable carrier.
- 50

Patentansprüche

1. Tretazicar zur Verwendung beim Bekämpfen einer parasitären Protozoeninfektion eines Tiers.
- 55 2. Verwendung von Tretazicar bei der Herstellung eines Medikaments zur Bekämpfung einer parasitären Protozoeninfektion eines Tiers.

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3. Verwendung nach Anspruch 2 oder Tretazicar zur Verwendung nach Anspruch 1, wobei das Tier ein Säugetier ist.
4. Verwendung oder Tretazicar zur Verwendung nach Anspruch 3, wobei das Säugetier ein Mensch ist.
- 5 5. Verwendung oder Tretazicar zur Verwendung nach einem der vorangehenden Ansprüche, wobei der Parasit, der die Infektion verursacht, mit einem Enzymsystem verbunden ist, das in der Lage ist, Tretazicar in eine zytotoxische Form zu aktivieren.
- 10 6. Verwendung oder Tretazicar zur Verwendung nach Anspruch 5, wobei das Enzym eine Nitroreduktase ist.
7. Verwendung oder Tretazicar zur Verwendung nach einem der vorangehenden Ansprüche, wobei das Tier ein solches ist, das endogen kein Enzymsystem enthält, welches Tretazicar in solchem Ausmaß aktiviert, dass unakzeptable Toxizität gegenüber dem Tier verursacht wird.
- 15 8. Verwendung oder Tretazicar zur Verwendung nach einem der vorangehenden Ansprüche, wobei der Parasit, der die Infektion verursacht, gegenüber Antibiotika resistent ist.
- 20 9. Verwendung nach Anspruch 2 oder Tretazicar zur Verwendung nach Anspruch 1, wobei die parasitische Infektion des Tiers durch einen oder mehrere der folgenden Parasiten verursacht ist: *Tiypanosoma cruzi*, *T. brucei*, *Leishmania spp.* insbesondere *L. infantum*, *Cryptosporidium* und *Giardia spp.*.
10. Tretazicar zur Verwendung nach Anspruch 1, wobei dem Tier auch eine oder mehrere andere Verbindungen verabreicht wird/werden, von denen bekannt ist, dass sie eine parasitäre Infektion bekämpfen.
- 25 11. Verwendung einer Kombination von Tretazicar und einer oder mehrerer Verbindungen, von der/denen bekannt ist, dass sie beim Bekämpfen einer Protozoenparasiteninfektion eines Tiers nützlich ist/sind, bei der Herstellung eines Medikaments zum Bekämpfen einer parasitären Infektion eines Tiers.
- 30 12. Verwendung von Tretazicar bei der Herstellung eines Medikaments zum Bekämpfen einer Protozoenparasiteninfektion eines Tiers, wobei dem Tier eine oder mehrere andere Verbindungen verabreicht wird/werden, von der/denen bekannt ist, dass sie nützlich beim Bekämpfen einer parasitären Infektion eines Tiers ist/sind.
- 35 13. Verwendung einer oder mehrerer Verbindungen, von der/denen bekannt ist, dass sie beim Bekämpfen einer Protozoenparasiteninfektion eines Tiers nützlich ist/sind, bei der Herstellung eines Medikaments zum Bekämpfen einer parasitären Infektion bei einem Tier, wobei dem Tier Tretazicar verabreicht wird.
- 40 14. Kombination von Tretazicar und einer oder mehrerer anderer Verbindungen, von der/denen bekannt ist, dass sie beim Bekämpfen einer Protozoenparasiteninfektion eines Wirtsorganismus nützlich ist/sind.
15. Kombination nach Anspruch 14, wobei der Wirtsorganismus ein Tier ist.
16. Kombination nach Anspruch 15 zur Verwendung in der Medizin.
- 45 17. Pharmazeutische Zusammensetzung, die die Kombination, wie sie in Anspruch 15 definiert ist, und einen pharmazeutisch akzeptablen Träger aufweist.

Revendications

- 50 1. Trétazicar pour une utilisation dans la lutte contre une infection parasitaire protozoaire chez un animal.
2. Utilisation du trétazicar dans la fabrication d'un médicament pour lutter contre une infection parasitaire protozoaire chez un animal.
- 55 3. Utilisation selon la revendication 2, ou trétazicar pour une utilisation selon la revendication 1, où l'animal est un mammifère.
4. Utilisation ou trétazicar pour une utilisation selon la revendication 3, où le mammifère est un être humain.

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5. Utilisation ou trétazicar pour une utilisation selon l'une quelconque des revendications précédentes, où le parasite provoquant l'infection est associé à un système enzymatique apte à activer le trétazicar en une forme cytotoxique.
6. Utilisation ou trétazicar pour une utilisation selon la revendication 5, où l'enzyme est une nitroréductase.
7. Utilisation ou trétazicar pour une utilisation selon l'une quelconque des revendications précédentes, où l'animal est un animal qui ne contient pas un système enzymatique endogène qui active le trétazicar à un degré qui entraîne une toxicité inacceptable pour l'animal.
8. Utilisation ou trétazicar pour une utilisation selon l'une quelconque des revendications précédentes, où le parasite provoquant l'infection est résistant aux antibiotiques.
9. Utilisation selon la revendication 2 ou trétazicar pour une utilisation selon la revendication 1, où l'infection parasitaire chez l'animal est provoquée par l'un quelconque ou plusieurs parmi les parasites suivants : *Trypanosoma cruzi*, *T. brucei*, *Leishmania spp.*, en particulier *L. infantum*, *Cryptosporidium* et *Giardia spp.*
10. Trétazicar pour une utilisation selon la revendication 1, où l'on administre aussi à l'animal un ou plusieurs autres composés connus pour être utiles dans la lutte contre une infection parasitaire.
11. Utilisation d'une combinaison de trétazicar et d'un ou plusieurs autres composés connus pour être utiles dans la lutte contre une infection parasitaire protozoaire chez un animal, dans la fabrication d'un médicament pour lutter contre une infection parasitaire chez un animal.
12. Utilisation du trétazicar dans la fabrication d'un médicament pour lutter contre une infection parasitaire protozoaire chez un animal, où l'on administre à l'animal un ou plusieurs autres composés connus pour être utiles dans la lutte contre une infection parasitaire chez un animal.
13. Utilisation d'un ou plusieurs autres composés connus pour être utiles dans la lutte contre une infection parasitaire protozoaire chez un animal, dans la fabrication d'un médicament pour lutter contre une infection parasitaire chez un animal, dans laquelle l'on administre du trétazicar à l'animal.
14. Combinaison de trétazicar et d'un ou plusieurs autres composés connus pour être utiles dans la lutte contre une infection parasitaire protozoaire chez un organisme hôte.
15. Combinaison selon la revendication 14, dans laquelle l'organisme hôte est un animal.
16. Combinaison selon la revendication 15 pour une utilisation en médecine.
17. Composition pharmaceutique comprenant la combinaison telle que définie dans la revendication 15 et un véhicule pharmaceutiquement acceptable.

Figure 1.

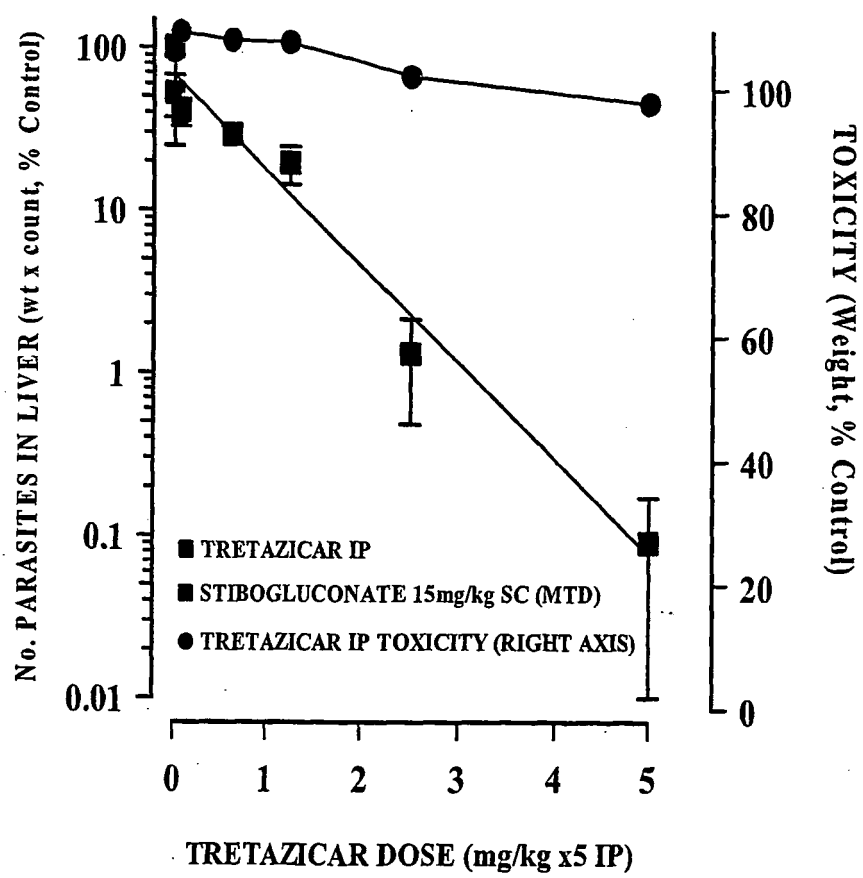


Figure 2.

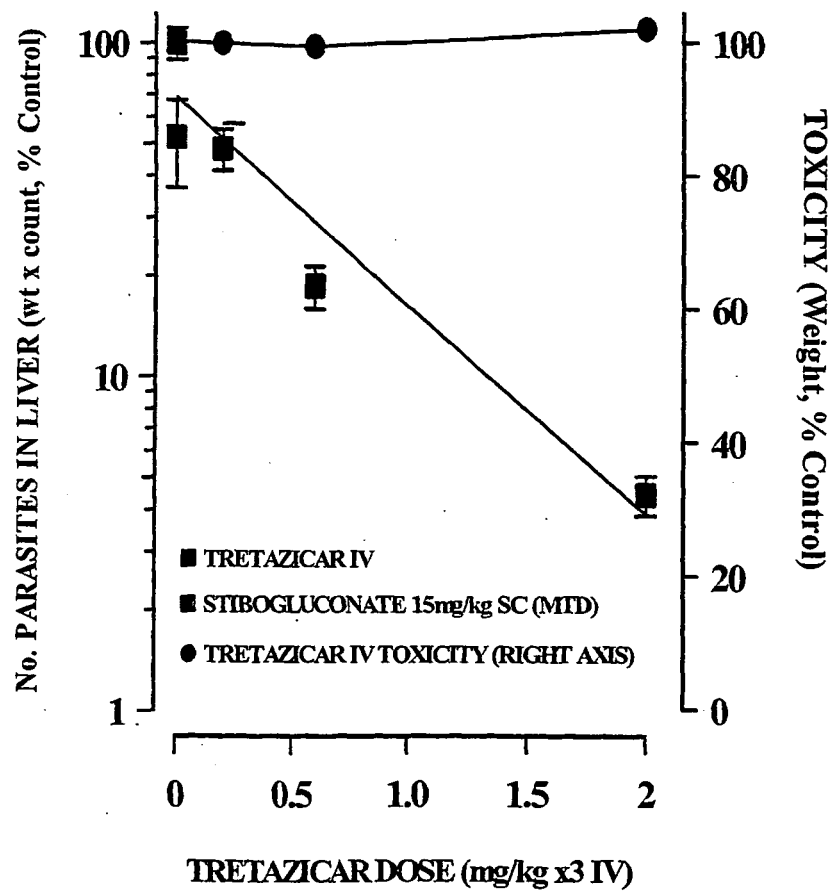
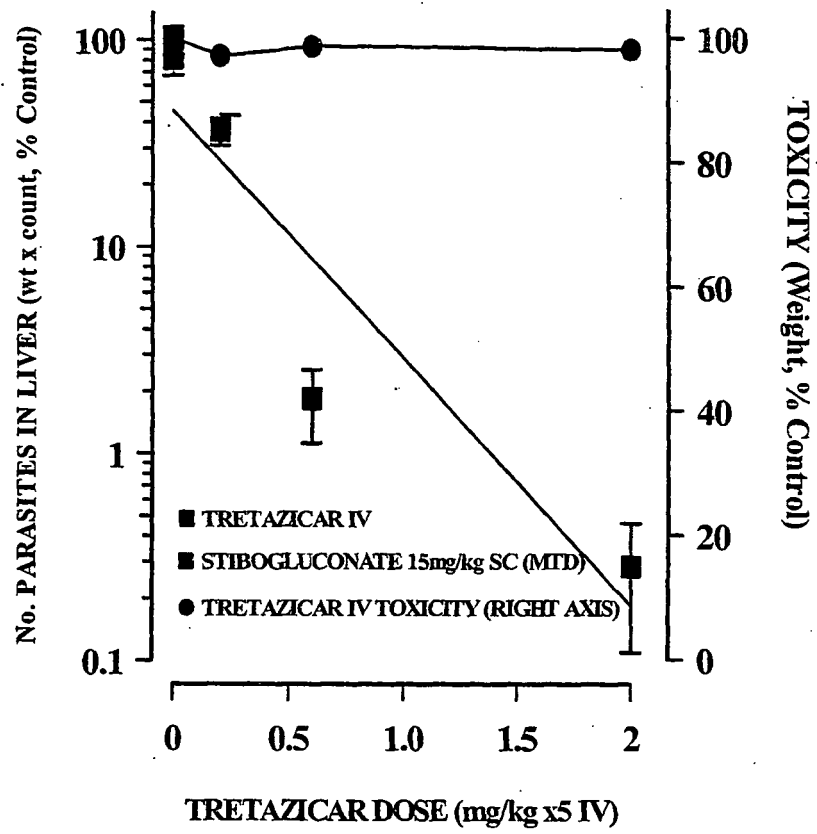


Figure 3.



REFERENCES CITED IN THE DESCRIPTION

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